

Supporting Information

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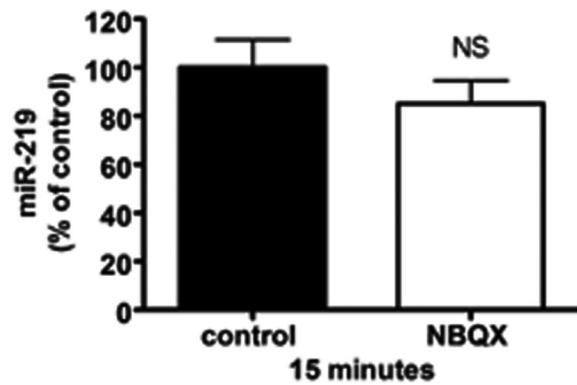


Fig. S1. Lack of effect of NBQX on miR-219 in mice. We assessed the regulation of miR-219 by acute administration of mice with a nonpsychotomimetic agent, and non-NMDA-R antagonist, NBQX. Adult male C57BL6 mice were injected with saline ($n = 3$) or NBQX (1 mg/kg; i.p. injection; $n = 3$; AMPA receptor antagonist) for 15 min. Real-time PCR analysis revealed that NBQX treatment did not significantly alter miR-219 expression in the PFC. NS, not significant; $P > 0.05$.

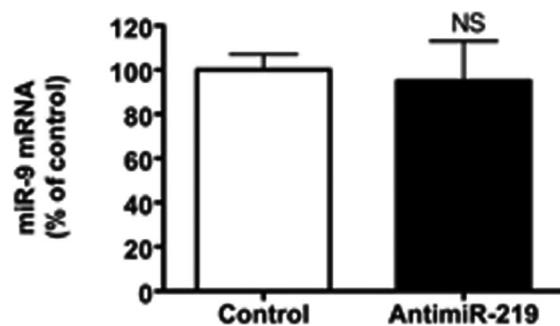


Fig. 52. miR-9 expression is not affected in LNA-antimiR-219 treated P19 cells. To examine whether LNA-based antagonism of miR-219 has nonspecific effects on microRNA regulation, we evaluated the expression of miR-9, a brain-enriched microRNA, in LNA treated cells. P19 cells were differentiated into a neuronal lineage with retinoic acid and cells were subsequently transfected with vehicle or 30 nM of antimiR-219 ($n = 6$ for each) for 48 h. RNA was isolated posttransfection and reverse-transcribed into cDNA for analysis of miR-9 expression by real-time PCR. MiR-9 levels were not significantly changed in LNA-antimiR-219 transfected cells. NS, not significant; $P > 0.05$.

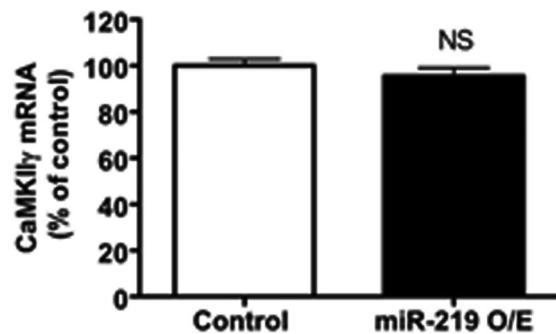


Fig. S3. CaMKII γ is a posttranscriptional target of miR-219 in neuronal cells. We examined the regulation of CaMKII γ mRNA and protein levels by miR-219 overexpression in differentiated P19 cells. After neuronal differentiation, P19 cells were transfected with vehicle or 30 nM of miR-219 RNA (Ambion) ($n = 6$ for each) for 48 h. RNA was isolated posttransfection and reverse-transcribed into cDNA for analysis of CaMKII γ expression by real-time PCR. CaMKII γ mRNA expression was not significantly changed in miR-219 transfected cells.

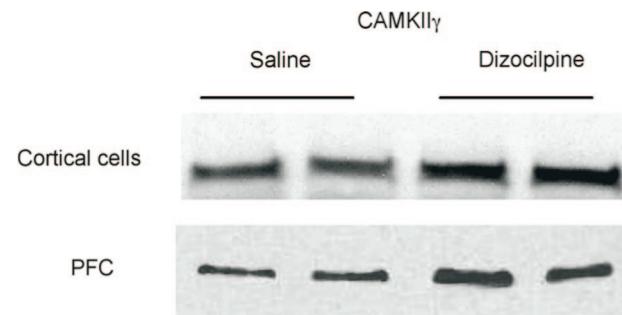


Fig. S4. CaMKII γ protein levels are up-regulated by dizocilpine. We examined the regulation of CaMKII γ protein levels by dizocilpine in vivo (0.5 mg/kg for 3 h; $n = 4$ each for saline and dizocilpine treated animals) and in cortical cells (treated for 18 h with 50 μ M dizocilpine; $n = 4$ each for saline and dizocilpine treated cells). Protein was extracted from either the prefrontal cortex of the mice or from primary cortical cells for Western blot analysis. CaMKII γ protein expression was increased both in vitro and in vivo, suggesting this kinase is one potential signaling mechanism for dizocilpine-mediated NMDA-R antagonism.

Table S1. Microarray analysis of microRNA expression in the mouse prefrontal cortex in response to dizolcipine (MK-801) treatment

Rank	Sanger 9 name	NCode2 probe ID	MicroRNA sequence	MK801 vs. control fold-change
1	mmu-miR-219	1426	UGAUUUGUCCAAACGCAAUUCU	0.22
2	mmu-let-7c	1268	UGAGGUAGUAGGUUGUAUGGUU	1.82
3	mmu-miR-150	1385	UCUCCCAACCCUUGUACCAGUG	1.80
4	mmu-let-7f	2066	UGAGGUAGUAGAUUGUAUAGU	1.71
5	mmu-miR-486	2209	UCCUGUACUGAGCUGCCCCGAG	1.82
6	mmu-let-7e	1421	UGAGGUAGGAGGUUGUAUAGU	2.27
7	mmu-let-7 g	1432	UGAGGUAGUAGUUUGUACAGU	1.67
8	mmu-miR-483	1844	UCACUCUCUCCCCUCCGUCUUGU	1.64
9	mmu-miR-7	2261	UGGAAGACAUAGUGAUUUUUGU	1.71
10	mmu-let-7d	1085	AGAGGUAGUAGGUUGCAUAGU	1.64
11	mmu-miR-153	2323	UUGCAUAGUCACAAAAGUGAUC	0.60
12	mmu-miR-361	2296	UUAUCAGAAUCUCCAGGGGUAC	1.52
13	mmu-miR-15b	1313	UAGCAGCACAUCAUGGUUUACA	1.54
14	mmu-miR-298	1084	GGCAGAGGAGGGCUGUUCUCC	0.62
15	mmu-let-7a	1428	UGAGGUAGUAGGUUGUAUAGU	2.35
16	mmu-miR-379	2276	UGGUAGACUAUGGAACGUAGG	1.52
17	mmu-miR-805	2852	GAAUUGAUCAGGACAUAGGG	1.51
18	mmu-miR-674*	1828	CACAGCUCCCAUCUCAGAACAA	1.42
19	mmu-miR-705	2270	GGUGGGAGGUGGGUGGGCA	1.91
20	mmu-miR-669c	1923	AUAGUUUGUGUGUGAUGUGUGU	1.48
21	mmu-let-7b	1431	UGAGGUAGUAGGUUGUGUGGUU	1.44
22	mmu-miR-204	1489	UUCCCUUUGUCAUCCUAUGCUG	1.49
23	mmu-miR-26a	2299	UUCAAGUAAUCCAGGAUGGC	1.40
24	mmu-miR-652	1961	AAUGGCGCCACUAGGGUUGUGCA	1.39
25	mmu-let-7i	2244	UGAGGUAGUAGUUUGUGUGUGU	1.39
26	mmu-miR-485-5p	1863	AGAGGCCUGCCCGUGAUAUJC	1.48
27	mmu-miR-676	2020	CCGUCCUGAGGUUGUGAGCU	1.44
28	mmu-miR-98	1423	UGAGGUAGUAAGUUGUAUUGU	1.48
29	mmu-miR-181c	1015	AACAUUCAACCUGUCGGUGAGU	0.73
30	mmu-miR-30a-3p	1505	CUUUCAGUCGGAUGUUUUGCAGC	1.39
31	mmu-miR-370	2258	GCCUGCUGGGGUGGAACCUGUU	0.74
32	mmu-miR-9*	1232	UAAAAGCUAGAUAAACCGAAAGU	1.38
33	mmu-miR-410	1809	AAUUAUACACAGAUGGCCUGUU	1.39
34	mmu-miR-672	2067	UGAGGUUGGUGUACUGUGUGUG	1.35
35	mmu-miR-191	1017	CAACGGAAUCCCAAAGCAGCU	1.36
36	mmu-miR-126-3p	1380	UCGUACCUGAGUAAAUAUGC	1.34
37	mmu-miR-341	1371	UCGAUCGGUCGGUCGGUCAGU	1.33
38	mmu-miR-151	1305	CUAGACUGAGGCUCCUUGAGG	1.44
39	mmu-miR-101b	1298	UACAGUACUGUGAUAGCUGAAG	0.75
40	mmu-miR-203	1398	UGAAAUGUUUAGGACCACUAG	1.33
41	mmu-miR-329	1012	AACACACCCAGCUACCUUUUU	1.31
42	mmu-miR-696	2021	GCGUGUGCUUGCUGUGGG	1.44
43	mmu-miR-542-5p	2215	CUCGGGGAUCAUAGUGACAG	0.76
44	mmu-miR-301	1103	CAGUGCCAUAAGUAAUGUCAAAGC	0.77
45	mmu-miR-125b	1182	UCCCUGAGACCCUAACUUGUGA	1.31
46	mmu-miR-9	2228	UCUUGGGUAUCAUGCUGUAUG	1.40
47	mmu-miR-323	1154	GCACAUUACACGGUCGACCUU	0.75
48	mmu-miR-29b	1869	UAGCACCAUUUAGAAAUCAGUGU	0.76
49	mmu-miR-128b	1153	UCACAGUGAACCGGUCUUCUUC	1.30
50	mmu-miR-149	1392	UCUGGCUCCGUGUCUUCACUCC	1.39
51	mmu-miR-101a	1297	UACAGUACUGUGAUAAACUGAAG	0.76
52	mmu-miR-15a	1312	UAGCAGCACAUAAUGGUUGUG	0.74
53	mmu-miR-24	1044	UGGCUCAGUUCAGCAGGAACAG	1.29
54	mmu-miR-539	2080	GGAGAAAAUUAUCUUGGUGUGU	0.78
55	mmu-miR-25	1139	CAUUCACUUGUCUCGGUCUGA	1.26
56	mmu-miR-125a	1193	UCCCUGAGACCCUUUAACCUGUG	1.28
57	mmu-miR-382	1806	GAAGUUGUUCUGGGUGGGAUUCG	1.31
58	mmu-miR-484	2191	UCAGGCUCAGUCCCCUCCCGAU	1.27
59	mmu-miR-181a	1172	AAACAUUCAACGCUGUCGGUGAGU	0.80
60	mmu-miR-449b	1886	AGGCAGUGCAUUGCUGCUGG	1.25
61	mmu-miR-674	1991	GCACUGAGAUGGGAGUGGUGUA	0.78
62	mmu-miR-485-3p	1906	AGUCAUACACGGCUCUCCUC	0.73
63	mmu-miR-434-3p	2338	UUUGAACCAUCACUCGACUCC	1.24
64	mmu-miR-744	2862	UGCGGGCUAGGGCUAACAGC	0.74

Rank	Sanger 9 name	NCODE2 probe ID	MicroRNA sequence	MK801 vs. control fold-change
65	mmu-miR-26b	1484	UUCAAGUAUUCAGGAUAGGUU	1.22
66	mmu-miR-335	1146	UCAAGAGCAUAACGAAAAAUGU	1.23
67	mmu-miR-541	1896	AAGGGAUUCUGAUGUUGGGCACA	1.21
68	mmu-miR-132	1014	UAACAGCUACAGCCAUGGUCG	1.23
69	mmu-miR-138	1089	AGCUGGUGUUGUGAAC	0.81
70	mmu-miR-760	2868	GAAAUCGGCUCUGGGUCUGGGGG	1.25
71	mmu-miR-27a	1485	UUCACAGUGGCUAAGUCCGC	0.82
72	mmu-miR-19b	1039	UGUGCAAUCCAUGCAAAACUGA	1.21
73	mmu-miR-194	1416	UGUACAGCAACUCCAUGGGA	1.25
74	mmu-miR-139	1384	UCUACAGUGCACGUGUCU	1.30
75	mmu-miR-181b	1830	AAACAUUCAUUGCUGUCGGGG	0.84
76	mmu-miR-148b	1362	UCAGUGCAUCACAGAACUUUGU	0.81
77	mmu-miR-100	1064	AACCCGUAGAUCCGAACUUGUG	1.24
78	mmu-miR-124a	2124	UAAGGCACGCCGGUGAACGCC	1.19
79	mmu-miR-330	1003	GCAAAGCACAGGCCUGCAGAGA	1.19
80	mmu-miR-23a	1114	AUCACAUUGCCAGGGAUUCC	1.18
81	mmu-miR-344	1118	UGAUCUAGCCAAGGCCUGACUGU	0.85
82	mmu-miR-93	1029	CAAAGUGCUGUUCGUGCAGGUAG	1.19
83	mmu-miR-487b	1816	AAUCGUACAGGGUCAUCCACU	0.80
84	mmu-miR-129-5p	1513	CUUUUUGCGGUCUGGGCUUGCU	1.19
85	mmu-miR-34a	1235	UGGCAGUGUCUUAGCUGGUUGUU	0.84
86	mmu-miR-690	1763	AAAGGCUAGGCUCACAACAAA	1.23
87	mmu-miR-129-3p	1021	AAGCCCUCUACCCCCAAAAGCAU	1.19
88	mmu-miR-380-3p	2178	UAUGUAGUAUGGUCCACAUUU	1.17
89	mmu-miR-23b	1930	AUCACAUUGCCAGGGAUUACC	1.18
90	mmu-miR-22	1022	AAGCUGCCAGUUGAAGAACUGU	1.19
91	mmu-miR-146	1409	UGAGAACUGAAUUCCAUGGUU	1.17
92	mmu-miR-30e*	2052	CUUUCAGUCGGAUGUUUACAG	1.16
93	mmu-miR-16	1272	UAGCAGCACGUAAAUAUUGCG	1.17
94	mmu-miR-433-3p	1937	AUCAUGAUGGCCUCCUCGGUGU	1.17
95	mmu-miR-691	1970	AUUCUGAAGAGAGGCAGAAAAA	1.19
96	mmu-miR-30d	1251	UGUAAACAUCCCGACUGGAAG	1.15
97	mmu-miR-148a	1361	UCAGUGCACUACAGAACUUUGU	0.87
98	mmu-miR-143	1415	UGAGAUGAAGCACUGUAGCUA	0.86
99	mmu-miR-543	1754	AAACAUUCGCCGGUGCACUUCU	1.19
100	mmu-miR-154	1101	UAGGUUAUCCGUGUUGCCUUCG	0.86
101	mmu-miR-185	1451	UGGAGAGAAAGGCAGUUC	1.21
102	mmu-miR-300	1126	UAUGCAAGGGCAAGCUCUUC	0.87
103	mmu-miR-146b	2237	UGAGAACUGAAUUCCAUAGGCU	1.15
104	mmu-miR-383	2069	AGAUCAGAACGGGACUGUGGU	1.16
105	mmu-miR-770-3p	2866	CGUGGGCCUGACGUGGAGCUGG	0.86
106	mmu-miR-137	1339	UUAUUGCCTAAAGAAUACCGCUAG	1.14
107	mmu-miR-497	1870	CAGCAGCACACUGUGGUUUGUA	0.86
108	mmu-miR-30a-5p	1460	UGUAACAUCCUCGACUGGAG	0.86
109	mmu-miR-424	1994	CAGCAGCAUUCAUGUUUUUGA	1.14
110	mmu-miR-31	1092	AGGCAAGAUGCUGGCAUAGCUG	0.87
111	mmu-miR-134	1470	UGUGACUGGUUGACCAGAGGG	0.88
112	mmu-miR-107	1163	AGCAGCAUUGUACAGGGCUA	0.88
113	mmu-miR-324-3p	1082	CCACUGCCCCAGGUGCUGCUGG	0.85
114	mmu-miR-409	1966	GAAUGUUGCUCGGUGAACCCUU	1.13
115	mmu-miR-350	2187	UUCACAAAGCCCAUACACUUCA	0.88
116	mmu-miR-223	1467	UGUCAGUUUGUCAAAUACCCC	1.13
117	mmu-miR-212	1288	UAACAGUCUCCAGUCACGGCC	0.89
118	mmu-miR-532	1959	CAUGCCUUGAGUGUAGGACCGU	0.88
119	mmu-miR-667	2233	UGACACCUGCCACCCAGCCCAAG	1.11
120	mmu-miR-145	1168	GUCCAGUUUUUCCAGGAAUCCUU	0.90
121	mmu-miR-128a	1350	UCACAGUGAACCGGUUCUUUU	0.90
122	mmu-miR-106b	1282	UAAAGUGCUGACAGUGCAGAU	1.11
123	mmu-miR-103	1164	AGCAGCAUUGUACAGGGCUA	1.11
124	mmu-miR-221	1088	AGCUACAUUGUCUGCUGGGUUU	0.90
125	mmu-miR-27b	2303	UUCACAGUGGCUAAGUUCUGC	1.11
126	mmu-miR-187	1381	UCGUGUCUUGUGUUGCAGCCG	1.17
127	mmu-miR-423	1874	AGCUCGGUCUGAGGCCUCAG	0.91
128	mmu-miR-668	2287	UGUCACUCGGCUCGGCCCACUACC	1.10
129	mmu-miR-467b	1925	AUAUACAUACACACACCAACAC	1.14

Rank	Sanger 9 name	NCode2 probe ID	MicroRNA sequence	MK801 vs. control fold-change
130	mmu-miR-346	1389	UGUCUGCCCGAGUGCUCUCCUCU	1.09
131	mmu-miR-337	1357	UUCAGCUCCUAUAUGAUGCCUUU	0.92
132	mmu-miR-501*	1818	AAUGCACCCGGGCAAGGAUUUG	0.92
133	mmu-miR-99b	1063	CACCCGUAGAACCGACCUCUUGCG	0.92
134	mmu-miR-338	1174	UCCAGCAUCAGUGAUUUUGUUGA	0.91
135	mmu-miR-342	1349	UCUCACACAGAAAUCGCACCCGUC	1.08
136	mmu-miR-28	1024	AAGGAGCUCACAGUCUAUUGAG	1.08
137	mmu-miR-29a	2154	UAGCACCAUCUGAAUUCGGUU	1.08
138	mmu-miR-99a	1066	ACCCGUAGAUCCGAUCUUGU	0.92
139	mmu-miR-495	1752	AAACAAACAUGGUGCACUUCUU	1.07
140	mmu-miR-592	2332	AUUGUGUCAAUAUGCGAUGAUGU	1.08
141	mmu-miR-17-5p	1031	CAAAGUGCUUACAGUGCAGGUAGU	1.08
142	mmu-miR-299	1458	UGGUUUACCGUCCCACAUACAU	1.08
143	mmu-miR-106a	1144	CAAAGUGCUAACAGUGCAGGUA	1.07
144	mmu-miR-324-5p	1938	CGCAUCCCCUAGGGCAUUGGUG	0.94
145	mmu-miR-376b	1936	AUCAUAGAGGAACAUCCACUUU	1.06
146	mmu-miR-200b	1042	UAAUACUGCCUGGUAAUGAUGAC	0.85
147	mmu-miR-140	2008	CAGUGGUUUUACCCUAUGGUAG	0.94
148	mmu-miR-706	1862	AGAGAAACCCUGUCUCAAAAAAA	0.93
149	mmu-miR-21	1315	UAGCUUAUCAGACUGAUGUUGA	1.05
150	mmu-miR-130a	2005	CAGUGCAUGGUAAAAGGGCAU	0.95
151	mmu-miR-381	2171	UAUACAAGGGCAAGCUCUCUGU	1.05
152	mmu-miR-433-5p	2149	UACGGUGAGCCUGUCAUUAUUC	1.05
153	mmu-miR-20a	1007	UAAAGUGCUUUAUGUGCAGGUAG	1.05
154	mmu-miR-199a*	1124	UACAGUAGUCUGCACAUUUGGU	0.94
155	mmu-miR-455-3p	1998	AUGCAGUCCACGGGCAUAUACACU	1.06
156	mmu-miR-222	1198	ACCUACAUUCGGCUACUGGUCUC	0.96
157	mmu-miR-152	2007	UCAGUGCAUGACAGAACUUGGG	1.05
158	mmu-miR-29c	2155	UAGCACCAUUUGAAUUCGGU	0.96
159	mmu-miR-328	1455	CUGGCCUCUCUGCCCCUUCCGU	1.06
160	mmu-miR-30c	1252	UGUAACACUCCUACACUCUCAGC	0.96
161	mmu-miR-467a	1926	AUAUACAUACACACACCUACAC	1.04
162	mmu-miR-365	2138	UAAUGCCCCUAAAAACCUUUAU	1.04
163	mmu-miR-195	1311	UAGCAGCACAGAAAUAUUGGC	0.96
164	mmu-miR-422b	2264	CUGGACUUGGAGUCAGAAGGCC	0.96
165	mmu-miR-434-5p	2077	AGCUCGACUCAUGGUUUGAAC	0.97
166	mmu-miR-30b	2280	UGUAAACACUCCUACACUCAGCU	1.03
167	mmu-miR-369-5p	1866	AGAUCGACCGUGUUUAUUCG	1.03
168	mmu-miR-340	1187	UCCGUCUCAGUUACUUUAUAGCC	0.98
169	mmu-miR-451	1755	AAACCGUACCAUUAUCAGAGU	1.03
170	mmu-miR-218	1143	UUGUGCUUGAUCUAACCAUGU	0.98
171	mmu-miR-320	1005	AAAAGCUGGGUUGAGAGGGCGAA	1.02
172	mmu-miR-127	2214	UCGGAUCCGUCUGAGCUUGGC	1.02
173	mmu-miR-376a	1945	AUCGUAGAGGAAAUCCACGU	1.02
174	mmu-miR-92	1335	UAUUCACUUGUCGCCGGCUG	0.98
175	mmu-miR-709	1885	GGAGGCAGAGGCAGGAGGA	0.99
176	mmu-miR-669a	1915	AGUUGUGUGUGCAUGUCAUGU	1.01
177	mmu-miR-496	2248	UGAGUAUUAUACAUUCCAAUCUC	0.99
178	mmu-miR-376b*	2106	GUGGAUUAUCCCUUCAUGGUU	0.99
179	mmu-miR-720	1947	AUCUCGUCGGGGCCUCUA	0.99
180	mmu-miR-181a*	1179	ACCAUCGACCGUUGAUUUAUCC	1.00
181	mmu-let-7d*	1323	CUAUACGACCUCUGCCUUUCU	1.00
182	mmu-miR-140*	2143	UACCACAGGGUAGAACACCGGA	1.00

Mouse brains were rapidly removed 15 min after injection with a psychotomimetic dose of MK-801 (0.5 mg/kg, i.p.) or saline, and RNA was extracted from the PFC region of the brain from all animals. RNA was pooled from each treatment group prior to purification of the short RNAs and tagging cDNA for microarray analysis from 2 separate pooled sets each for saline controls and MK-801-treated animals ($n = 4$ animals per pool). Intensities shown represent background-subtracted median spot intensities further processed by log2 transformation and normalization. For normalization, the average spot intensity of mouse microRNA probes with detectable signal on each array (norm factor) was determined, this norm factor was subtracted from each spot intensity, and the average of the norm factors for the 4 arrays were added back to each probe intensity. This experiment was repeated in the exact same manner and the fold-change data are the mean of the 2 sets of experiments.

*MicroRNA probes with intensity above a detection threshold ($T = \text{mean negative control signal} + 5 \text{ standard deviation of negative controls}$) in 4 of 4 samples hybridized to the Ncode 2 probe set (Invitrogen) are listed, along with maximum spot intensities from duplicate spots for each probe in each sample.

Table S2. Bioinformatic identification of mRNA targets for miR-219

Gene symbol	Gene name	RNA22 energy value
FOXJ3	forkhead box J3	-31.3
TACC1	transforming, acidic coiled-coil containing protein 1	-24.2
CaMK2G	calcium/calmodulin-dependent protein kinase II	-24
OTX2	bicoid class homeodomain protein	-23.9
ZFP238	zinc finger protein 238	-20.8
PDGFRA	platelet derived growth factor receptor alpha	-20.3

To identify the strongest putative mRNA targets for miR-219, the mRNA candidates using 4 different bioinformatic programs (miRanda, PicTar, TargetScan, RNA22) were combined. The list was narrowed to 6 of the top bioinformatic mRNA targets based on their identification by all 4 programs and exhibiting a minimum cutoff energy of -20. From this list, CaMK2G was found to be intricately associated with NMDA signaling.